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Foxtail grass (*Setaria viridis*)-induced ulcerative stomatitis-gingivitis resembling viral vesicular stomatitis in horses

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ABSTRACT

Physical trauma, dietary factors, toxins, immune-mediated disorders, and viral infections are known causes of equine stomatitis. There are also few reports of grass awn-associated stomatitis in horses. Movement of 323 horses was restricted at a Hungarian racetrack because of a suspected vesicular stomatitis (VS) outbreak. Many horses were affected at the same time, and an infectious disease or common offending source was suspected. To establish the nutritional origin of the feed, botanical examination and a food provocation test were carried out. Two healthy adult horses were fed exclusively with the hay of concern for a two-week period. All horses at the racetrack and the experimental horses were clinically examined daily and tested for VS, viral arteritis, glanders, and equine herpesviruses. Biopsy samples were taken from the oral lesions to characterize the histological alterations. Botanical analysis revealed that more than 15% of the forage was foxtail grass *(Setaria viridis)*. The nutrient content of the forage was adequate for horse maintenance, while its mold and yeast counts were below the reference limits. Competition horses showed signs of depression, decreased appetite, and drooling and ulceration of the lips, tongues, and gingiva after four hours of exposure to the tested forage or hay. The horses in the feeding trial showed the first clinical signs of oral papules on day four, and then showed rupturing pustules, ulcers, and extensive granulation tissues on day seven. Experimental horses did not show signs of depression or loss of appetite. Stomatitis healed spontaneously when the hay was changed. Results of the serological tests, hematological tests, biochemical analysis, and gastroscopy did not show significant alterations. Biopsy samples from both the competition and the experimental horses invariably showed grass awns surrounded by reactive mixed inflammatory cells and granulomatous inflammation. Since differential diagnosis of ulcerative stomatitis include highly contagious viral diseases like VS, quarantine measurements are necessary during stomatitis outbreaks. Differentiation based exclusively on clinical signs is not recommended, although wedged plant particles along the periodontal gingival sulcus might be indicative of inappropriate forage source. Evaluation of the feed in case of a stomatitis outbreak should be immediately performed. In case of grass awn-associated stomatitis, differences are noticed in the severity of lesions possibly based on plant types and plant percentage in the hay. As such, the extent to which foxtail grass-contaminated hay causes ulcerative gingivitis may be increasing because of recent changes in climate.

1. Introduction

Physical trauma, dietary factors, toxins, immune mediated disorders, and viral infections are known causes of stomatitis in horses (McCluskey and Mumford, 2000). In herbivores, coarse pasture and roughage can cause laceration or abrasions on the mucosa of the lips, oral cavity, gingiva, tongue, and oropharynx (Bankowski, 1956; McCluskey and Mumford, 2000; Mohammadi and Sardari, 2009). There are few reports of grass awn-associated stomatitis in horses, and some reports are anecdotal (Turnquist et al., 2001; Mohammadi and Sardari, 2009). Viral causes of ulceration in the oral cavity of horses include vesicular stomatitis virus (VS), equine viral arteritis, herpesvirus (Vengust et al., 2008), and horsepox virus (contagious pustular stomatitis) (Monreal et al., 1995; Letchworth, 1996; McCluskey and Mumford, 2000). Because of the open borders within the European Union and the speed with which the agent of an infectious disease can be disseminated through movement of goods and people, the importance of recognizing the cause of stomatitis in horses cannot be under-

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estimated (Bankowski et al., 1956). Since VS is a notifiable disease and Europe is considered free from outbreaks, immediate quarantine measures are implemented if occurrence is suspected.

In September 2012, the National Food Chain Safety Office in Hungary introduced quarantine measurements for horses staying at a Hungarian racetrack during an international equestrian event (horse-racing and eventing) to prevent the spread of stomatitis. Participants arrived from many countries outside of Europe, including the United States where VS is endemic (OIE, 2012). Movement of 323 horses was restricted because of suspected VS outbreak. Since many horses were affected at the same time, an infectious disease or a common offending source was suspected. Horses were provided with different sources of water and concentrates, but they were fed with the same hay. The objective of this study was to elucidate whether hay was an offending source of the ulcerative stomatitis outbreak.

2. Material and methods

The study was permitted by the Animal Health and Welfare Directorate of the National Food Chain Safety Office (NFCSO). All 323 horses at the racetrack were examined daily and horses with clinical signs were isolated. Blood samples were taken from the jugular vein and tested for VS with virus neutralization for Indiana and New Jersey strains, and for viral arteritis by virus neutralization, real time polymerase chain reaction (PCR) for equine herpes 1, 4, (IDEXX RealPCR™ Tests, IDEXX Laboratories, Germany), and 2, 5 (Nordengrahn et al., 2002) and for glanders with complement fixation test (Bioveta, Czech Republic) in the accredited central laboratory of the NFCSO. Biopsy samples were taken under sedation (xylazine 0.3 mg/kg and butorphanol 0.02 mg/kg) and local anesthesia with lidocaine (10%) from the gingival alterations of the maxilla of two competition horses to characterize the histological alterations. Samples were collected in 10% formalin and evaluated with haematoxylin-eosin stain.

To establish nutritional origin of the feed, botanical examination, hay analysis, and a food provocation test were carried out. Sampling of the large round bales for nutrient analysis and botanical examination was carried out by selecting 10 representative bales and collecting two cores from the circumference of each. To sample the center parts of the bales, we reached inside each bale and removed two or three handfuls from different locations. All samples were put in a bucket and homogenized. To identify the plant species of the samples, macroscopic and microscopic examinations were performed. Hay analysis included the determination of dry matter (DM), ash, ether extract, crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). These measurements were carried out according to relevant ISO standards (ISO 5983 – 16472)[1,2,3,4,5,6]. Digestible energy (DE) was estimated from the chemical composition. Mold and yeast counts were performed according to ISO 21527[7] standard.

The provocation test was performed during April and May 2013 at the Large Animal Clinic of Szent Istvan University, Faculty of Veterinary Science. Between September 2012 and April 2013, the forage samples were stored in a dry, covered location nearby to avoid being subjected to weathering. Two healthy Hungarian warmblood horses, one gelding and one mare, aged 10 and 15 years, respectively, were fed exclusively with the hay of concern for a two-week period. Horses were kept in a 20×30 m sandy paddocks during the day and they were moved to individual boxes with wood shavings as bedding material overnight. Water was offered as needed. The two horses were weighed before and after the feeding period. The animals were subjected to daily clinical examination from the first day until the end of the second week after the cessation of the feeding trial. Blood samples were taken from the jugular vein on the first and 14th day for hematological (Abacus Junior Vet, Diatron, Hungary) and biochemical analyses (Olympus AU640, Germany), and to perform serological tests for VS, viral arteritis, glanders, and PCR for equine herpesviruses (EHV 1, 2, 4, 5). On the 10th day, representative samples of ulcerative lesions were collected from the gingiva for histopathology. Biopsy samples were taken from the oral cavity as described above. Esophagoscopy and gastroscopy (CV100, Olympus, Germany) was performed immediately after the biopsy sampling under the same sedation. Following the trial, horses were fed with good quality grass hay. The healing process was followed in the next two weeks by daily clinical examinations.

3. Results

3.1. Evaluation of the hay

Results of botanical and nutrient content analyses are listed in Table 1. The foxtail grass *(Setaria viridis)* identified in the hay had a dense hairy seed head with long spikelets (Fig. 1.). The mold count of the hay sample was 8.2×10^4 cfu/g, yeast count was 1×10^5 cfu/g, and aerobic bacterial count was $< 1 \times 10^5$ cfu/g.

3.2. Clinical findings

Twenty nine (9%) adult horses of different breeds (9.9% of thoroughbreds and 6% of warmbloods) and gender (11% of males and 6.5% of females) showed signs of decreased appetite (86.2%), depression (75.8%), drooling (65.5%), and ulceration of the gingiva (86.2%), lips (79.3%), and tongues (20.6%) at the racetrack. Racehorses and competition horses in contact with the hay contaminated with foxtail grass showed clinical signs after four hours of exposure. The horses in the feeding trial weighed 530 kg and 590 kg at the beginning and 554 kg and 612 kg at the end of the trial, respectively. These horses showed the first clinical signs of oral papules, mucus membrane erythema, and plant particles wedged along the periodontal gingival sulcus on the 4th day (Fig. 2). Clinical signs quickly worsened: bleedings and erosions occurred on the 5th day, then rupturing pustules appeared, ulcers were formed, and extensive granulation tissues were observed by the 7th day (Fig. 3). Similar alterations were visible on the lateral aspect of the tongues and lips (Fig. 4). Experimental horses were in good general condition, i.e., they did not show any signs of depression or loss of appetite. Salivation was observed only at the end of the second week. Comparisons of clinical findings are listed in Table 2. Both racetrack and experimental horses spontaneously recovered within two weeks after changing the hay. Ten days after finishing provocative feeding, there were no obvious abnormalities in the oral cavity other than some scarring and loss of pigmentation at the site of previous ulcerations.

3.3. Results of serological and PCR examinations

All serological results of blood samples for VS, viral arteritis, and glanders, including the quarantined horses at the racetrack and the

[1] ISO 5983:2005. Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content. ISO, International Organisation for Standardization. http://www.iso.org

[2] ISO 5984:2002. Animal feeding stuffs – Determination of crude ash. ISO, International Organisation for Standardization. http://www.iso.org

[3] ISO 6492:1999 Animal feeding stuffs – Determination of fat content. ISO 6492:1999 Animal feeding stuffs – Determination of fat content

[4] ISO 6496:1999 Animal feeding stuffs – Determination of moisture and other volatile matter content. ISO, International Organisation for Standardization. http://www.iso.org

[5] ISO 13906:2008. Animal feeding stuffs – Determination of acid detergent fiber (ADF) and acid detergent lignin (ADL) contents. ISO, International Organisation for Standardization. http://www.iso.org

[6] ISO 16472. 2006. Animal feeding stuffs. Determination of amylase-treated neutral detergent fiber content (aNDF). ISO, International Organisation for Standardization. http://www.iso.org

[7] ISO 21527–2:2008. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 2: Colony count technique in products with water activity less than or equal to 0,95. ISO, International Organisation for Standardization. http://www.iso.org

Table 1
Results of botanical and nutrient content analyses.

	Sample	Medow hay (Geor et al., 2013)
Botanical analysis		
foxtail grass *(Setaria viridis)*	15%	
Kentucky bluegrass *(Poa pratensis)*	72–75%	
red fescue *(Festuca rubra)*	8–9%	
brown knapweed *(Centaurea jacea)*	1–2%	
yellow rattle *(Rhinanthus minor)*	1–2%	
Nutrient analysis		
DE_{horse} (MJ/kg)	9,71	7,6
CP (g/kg)	93,1	80
NDF (g/kg)	596	530
ADF (g/kg)	377	410

Fig. 1. The hay contained 15% of foxtail grass (*Setaria viridis*). Its seed head is a dense, hairy panicle 5–30 cm long with spikelets of 1.8 – 2.2 mm length.

Fig. 2. Mucus membrane erythema and plant particles wedged along the periodontal gingival sulcus on the 4th day.

horses involved in the trial were negative. Results of equine herpesvirus PCR examinations were also negative.

3.4. Results of further diagnostic tests

Results of complete blood cell count, biochemistry analysis, and gastroscopy of the caudal part of the oral cavity, pharynx, esophagus, and stomach did not show significant effects. Biopsy samples from both the competition and the experimental horses invariably showed grass awns surrounded by reactive mixed inflammatory cells (lymphocytes, histiocytes, and some neutrophils) and granulomatous inflammation (Fig. 5).

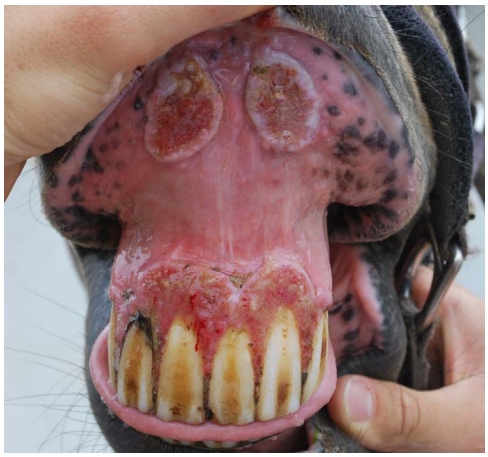

Fig. 3. Typical extensive granulation tissue with foxtail irritation.

Fig. 4. Local bleeding ulceration at the mucocutaneous junction of the lip.

Table 2
Comparisons of clinical findings.

	Horses on the racetrack	Horses of the provocation test
onset of clinical signs	4 h	4 days
clinical signs		
• loss of apetite	yes	no
• depression	yes	no
• drooling	4 h	14 days
• oral ulcers	yes	yes
• granulation tissue	no	yes

4. Discussion

To the best of our knowledge, this is the first feeding trial demonstrating that feeding hay of poor quality containing *S. viridis* could cause stomatitis in horses. We fully describe the pathophysiology of grass awn-caused ulcerative stomatitis that resembled VS.

Differential diagnosis of ulcerative stomatitis includes highly contagious viral diseases like VS. This virus causes erosions and ulcers on the horses' lips, palate, tongue, and also possibly on the prepuce, udder, and coronary bands, and the horse may develop fever (McCluskey and Mumford, 2000; Green, 1993). Protective measures are limited to quarantining infected horses and strictly limiting the means and conditions of transportation. Prompt differential diagnosis is essential to reduce the time of restricted movements of equidae. Incubation period varies from 2 to 8 days, and the average incubation is 3–5 days. Convalescent animals develop serotype-specific antibodies within 4–8 days of infection that can be detected by enzyme-linked immunosorbent assay (ELISA) or virus neutralization (VN) tests (OIE, 2012). All horses in the European Union should be seronegative for VS. Most of the

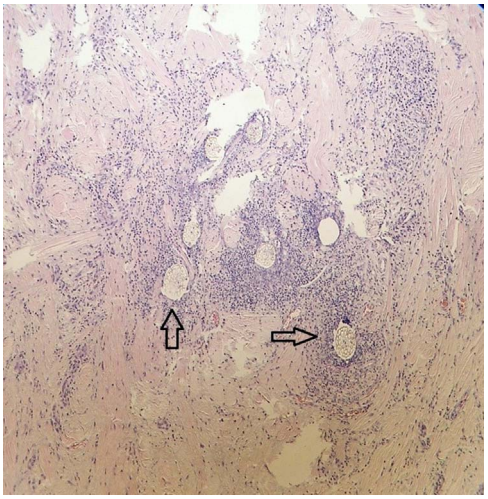

Fig. 5. Grass awns (arrows) surrounded by reactive mixed inflammatory cells and granulomatous inflammation (HE, 100*).

infected horses sampled 24–48 h after the appearance of the clinical signs should be seropositive. The horses in our study were quarantined for 10 days until all the tests for viral diseases were completed and showed negative results. Although there is no vesicular phase with foxtail irritation, VS cannot be excluded based on this because viral vesicles and fever might get unnoticed. Differentiation based exclusively on clinical signs is not recommended, although wedged plant particles along the periodontal gingival sulcus might be indicative of inappropriate forage source. Extensive granulation tissues that appear approximately one week after beginning of ingestion are suggestive of plant awn caused injuries. Examination and evaluation of horse feeds during stomatitis outbreaks should be immediately performed.

When evaluating stomatitis outbreaks at racetracks, causes of the outbreak other than viral infections, such as chemical, fungal, or blister beetle (*Epicauta spp*.) contamination of the hay should be excluded. Blister beetles mainly occur in alfalfa hay and cause irritation (blisters) in horse mouth and throughout their gastrointestinal tract. The cantharidin toxin produced by the beetles is excreted through the kidneys, causing urinary tract ulceration, inflammation, and severe toxicity, which can result in electrolyte changes (Helman and Edwards, 1997). Abdominal pain and neurologic signs, which are common during cantharidin toxicosis were absent in the present study. Slaframine toxicosis, caused by the ingestion of alfalfa hay contaminated by *Rhizoctonia leguminicola,* might cause similar clinical signs with drooling. The fungal infection is first seen as small black patches on the leaves, and then spreads to cover the entire plant (Riet-Correa et al., 2013). In the present study, the hay did not contain alfalfa or red clover; neither blister beetles nor black patches caused by fungal infection were found when the hay was microscopically examined. Some blistering chemical compounds could cause similar alterations on the oral mucous membrane, but such types of contamination of the hay could be excluded based on the history and physical examination of the feedstuff. In our study, the histopathologic results clearly demonstrated that the mucus membrane alterations were induced by the grass awns. It is known that ingestion of forage containing yellow bristle grass (*Setaria lutescens*), varieties of triticale, mouse barley (*Hordeum murinum*), and prairie foxtail (*Setaria geniculata*) cause characteristic oral ulceration in exposed horses in America, Australia, and Asia (Bankowski et al., 1956; McCosker and Keenan, 1983; Linnabary and Held, 1986; Turnquist et al., 2001; Johnson et al., 2012). In Europe, to the best of our knowledge, this was the first and largest outbreak caused by *S. viridis*.

Setaria viridis is a species of grass known by many common names, including green foxtail, green bristlegrass, and wild foxtail millet. *S. viridis* is a native of Europe but spread to North America as early as 1821 (Douglas et al., 1985), and now grows in most temperate countries of the Northern and Southern hemispheres (Clayton, 1980). *S. viridis* is reported to grow throughout Europe except the Azores (Portugal), the Faeroe Islands, Ireland, Iceland, and northern Russia, and it has been observed sporadically in the UK (Clayton, 1980). It grows mainly in cultivated fields and gardens, but also in waste places, disturbed areas, and along roads (Holm et al., 1977). The seed head is a dense hairy panicle 5–30 cm long with spikelets of 1.8–2.2 mm length. The morphology of these species allows them to cause a continual irritation of the mucosal membrane, contributing to the development of symptoms similar to those of VS.

According to previous studies describing stomatitis caused by plant awns (Linnabary and Held, 1986; Turnquist et al., 2001; Johnson et al., 2012) differences are noticed in the severity of clinical signs and the extent of injury possibly based on plant types and plant percentage in the hay. Foxtail grass do not cause lesions deeper in the oropharynx or other parts of the alimentary tract (Linnabary and Held, 1986; Turnquist et al., 2001; Johnson et al., 2012), which is in contrast to clinical signs of coughing due to pharyngitis described in the triticale report and the report describing mouse barley laceration on the soft palate (McCosker and Keenan, 1983; Mohammadi and Sardari, 2009). Mechanical pressure exerted during taking up and chewing of the hay is necessary to force the awns through the mucus membrane. It is likely that oral grinding and salivary softening of the plant awns make them less harmful when they reach the esophagus and stomach. The oral lesions described in our study disappeared spontaneously within 10 days after cessation of the feeding trial. This is in line with previous findings where stomatitis was caused by *Setaria geniculate* (Turnquist et al., 2001; Johnson et al., 2012). Contrary to this, Linnabary and Held (1986) found that stomatitis caused by *Setaria lutescens* did not disappear spontaneously within 5 months, and surgical debridement was necessary. This difference could be explained by the different seed head structure of the plant species. *S. lutescens* cause stomatitis in horses and cattle even with a contamination as low as 1.8% in the hay.

Nutrient and energy contents of the examined hay met the maintenance requirements of the experimental animals. Some authors have reported that hay contaminated with Setaris spp. is less palatable to horses and is rarely voluntarily eaten (Linnabary and Held, 1986), but in our feeding trial, the animals showed great appetite for *S. viridis* and gained weight. During the outbreak, competition animals were more sensitive with drooling and loss of appetite, which could result in weight loss and poor performance. Protracted storage of the hay might have caused softening of the awns and made them less harmful, but individual sensitivity cannot be excluded. Granulation tissues were not observed in the mouth cavity of the race- or sport-horses during the original outbreak, possibly because these horses showed feed refusal and then forage was changed immediately once oral ulcerations were detected.

The practice of feeding poor quality hay is often a direct consequence of the relative unavailability of better quality forage following periods of protracted drought (Johnson et al., 2012). *S. viridis* benefits from high temperatures and prolonged exposure to sunlight (Douglas et al., 1985), and it also has the potential to benefit from increasing levels of carbon dioxide (Ziska and Bunce, 1997). The problem caused by *S. viridis* is also now aggravated by the evolution of herbicide-resistant biotypes. Therefore, the extent to which foxtail grass contamination of hay causes ulcerative gingivitis in the European Union may be increasing because of environmental pollution and recent changes in climate.

Conflict of interest statement

I wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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